

NOV 08 '02 11:36 FR PATHOLOGY/LAB MED. 905 577 0198 TO  
SEN 61. VAN DYKE & ASSOCIATES, P.A.; 407 228 0329; NOV-7-02 4:24PM;P.02  
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DECLARATION OF JACK GAULDIE, Ph.D.  
 Examining Group 1635  
 Patent Application  
 Docket No. GDI-1CPA1  
 Serial No. 09/360,199

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner : Schnizer, Richard  
 Art Unit : 1635  
 Applicants : Gauldie et al.  
 Serial No. : 09/360,199  
 Docket No. : GDI-1CPA1  
 Filed : 07/23/1999  
 For : Intestinal Gene Therapy

Assistant Commissioner for Patents  
 Washington, D.C. 20231

DECLARATION OF JACK GAULDIE, Ph.D.

I, Jack Gauldie, Ph.D. hereby declare and say as follows:

THAT, I am employed as Professor and Chairman, Department of Pathology and Molecular Medicine at McMaster University, Hamilton, Ontario, Canada;

THAT, I earned my Ph.D. in Biological Chemistry in 1968 from University College, University of London UK, a copy of my curriculum vitae is attached hereto as Exhibit A;

THAT, I am one of the above-named Applicants and inventors of the subject matter described and claimed in the above-identified patent application;

THAT, by virtue of my educational and employment background, my attendance at seminars, my ongoing research, my continuing review of scientific periodicals and journals, and through correspondence with professional colleagues, I am aware of the level of skill of one ordinarily skilled in the art of immunology and vaccinology;

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THAT, I have studied the application Serial No. 09/360,199 and all office actions which have been issued during prosecution of this application (including cited references), as well as all responses which have been filed on the Applicants' behalf, and being thus duly qualified declare as follows:

1. The Office action questions whether the vaccination methods taught in the specification and claimed in the subject application achieve the desired therapeutic effect of immunizing an animal against a predetermined pathogen. The office action cites a number of references to allege that gene therapy and genetic immunization are unpredictable arts. In particular, the office action questions whether the cytotoxic T cell assay (CTL assay) is reliable enough to establish whether the claimed methods achieve a therapeutic effect. The office action cites Bachmann et al (1994) for the proposition that comparative studies have shown that CTL responses readily detectable after in vitro restimulation may not be detected by any in vivo assay. Citing Bachmann, the office actions states the following: "One should therefore be very cautious not to 'over-interpret' cytotoxicity found only by  $^{51}\text{Cr}$ -release after secondary in vitro restimulation; without in vivo confirmation the result may be biologically irrelevant." The office action further states that the "Applicant . . . has not provided sufficient evidence or reasoning to support the position that a protective immune response will be generated against any antigen by the claimed methods or composition." I respectfully disagree with the Examiner's position. Since the publication by Bachmann, a number of gene based vaccine approaches have been developed, primarily aimed at developing anti-tumor immunity, in which protection from tumor challenge is associated with the presence of CTL determined by in vitro secondary expansion of T cells and  $^{51}\text{Cr}$  CTL assays. Moreover, the details we now supply demonstrate direct protective effects of this immunization protocol and all are associated with CTL detection. There may be some instances, such as those quoted by Bachmann, in which CTL assays after secondary in vitro expansion do not correlate with protection, however, most data recently published show correlation, including the data supplied herein.

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2. Although I believe that the CTL assay example provided in the specification is sufficient to support a claim to therapeutic value in the present case, as further evidence, I provide herewith *in vivo* data from two different studies, which unquestionably demonstrate that the claimed methods do indeed immunize against targeted pathogens. These studies are provided as Exhibits B and C. Exhibit B demonstrates that Adenoviral-based gene delivery in the lower GI tract induces antigen-specific immune responses and protection from Tumour challenge, correlating with the presence of CTL positive reactions in spleen cells from immunized animals. Exhibit C demonstrates that Adenoviral based antigen gene delivery to rectal epithelium induces protective local immunity against HSV-2 infection, challenged either by vaginal or rectal administration of the pathogen. In view of these two studies, there can be no question that the claimed methods, as claimed in Applicants' most recent response filed September 15, 2002, are directed to a useful, therapeutic vaccination methodology.

3. The claims as pending before Applicants' September 15, 2002 amendment were rejected over Wang and Henning (PCT publication and U.S. patents). The amendments to the claims so distinguish the prior art that it cannot be said that the Wang and Henning references anticipate or render obvious the now pending claims. I have carefully reviewed the Wang and Henning references. The Examiner correctly asserts that "neither Wang nor Henning teach a working example of a therapeutic effect," see page 15, paragraph 2 of the last office action. The Henning references disclose a method of introducing nucleic acid into the intestine using naked DNA or using various viral vectors. Henning discloses a few hypothetical examples of introducing DNA into intestinal cells. I point out that none of the examples discuss the use of an adenoviral vector; they are limited to retrovirus vectors, which are of limited use *in vivo*. Furthermore, Henning provides no working example, either *in vitro* or *in vivo*, of a methodology that may act to immunize an animal. Based on the teachings of Henning, one skilled in the art is still left wondering whether cells can be transfected in the intestine *in vivo* to express a given a gene. One skilled in the art knows no more about whether a gene can be reproducibly expressed in the intestine, much less whether an animal can be immunized against a specific pathogen by expression of a given gene.

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There is simply no connection between the method of exposing intestinal cells to a nucleic acid taught by Henning and successfully expressing a gene, whereby such expression leads to a successful vaccination of an animal against a given pathogen. The subject application is the first demonstration, as far as I am aware, that shows successful introduction of a gene into genitourinary epithelial cells using an adenoviral vector, whereby a protein antigen is generated that induces an immune response.

4. With respect to the Wang et al. reference, it discloses a specific study involving the exposure of vaginal mucosa to a non-viral vector expressing HIV-1 envelope proteins. The study shows that exposure to the non-viral based DNA plasmid produces immunoglobulins that showed activity in the in vitro cell-free infection assay. The assay involved taking vaginal washes from treated and non-treated animals and combining the wash with HIV-1/MN cell-free virus. The cell free virus was then combined with MT-2 cells, and the ability of the virus to infect the cells was observed. In some cases, it does appear that something in the vaginal wash affects the ability of the virus to infect the MT-2 cells. It is conjectured by Wang et al. that it is immunoglobulins present in the vaginal wash that is affecting the ability of the cell-free virus to infect the MT-2 cells. This study provides little additional information over Henning as to whether a given viral vector is able to be introduced into mucosal cells, express a gene of interest, and induce a protective immune response against a given pathogen. There is the suggestion that it may be worthwhile to study different routes of administration using different vectors. However, in view of either Henning or Wang, it cannot be said that any given route of administration, using non viral or viral vectors, would vaccinate a treated animal with a reasonable expectation of success. The Applicants of the present application are the first to demonstrate that specific vaccination is achievable through gastrointestinal or genitourinary routes by application of an adenoviral vector encoding a specific antigen gene..

5. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information in belief are believed to be true; and further that these statements were made with the knowledge that willful false statements in the like so made are punishable by fine or imprisonment, or both, under

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§1001 of title 18 of the U.S.C. and that such willful false statements made jeopardize the validity of the application or of any patent issuing thereon.

Further declarant sayeth naught.



Jack Gauldie

Jack Gauldie, Ph.D.

Nov 8/02

Date

## Exhibit A: Dr. Jack Gauldie's *Curriculum Vitae*





## CURRICULUM VITAE DR. JACK GAULDIE

Date of Birth: November 14, 1942  
Place of Birth: Greenock, Scotland  
Nationality: Canadian  
Marital Status: Married, 2 children

Business Address: Department of Pathology and Molecular Medicine  
Room 2N16  
McMaster University  
1200 Main Street West  
Hamilton, Ontario  
Canada L8N 3Z5

Tel: (905) 521-2100 Ext. 76332  
FAX: (905) 577-0198  
Email: [gauldie@mcmaster.ca](mailto:gauldie@mcmaster.ca)

Home Address: 55 Bond Street South  
Hamilton, Ontario, Canada L8S 1S8  
Tel: (905) 529-3514

Degrees:

1964 B.Sc.	Chemistry, McMaster University, Hamilton, Ontario, Canada
1968 Ph.D.	Biological Chemistry, University College, London, England
1984 Fellow	Canadian Academy of Clinical Biochemistry
1995 Fellow	Canadian Academy of Clinical Biochemistry Fellow in Clinical Immunology - FCACB(I)

Awards:

1964	Chemistry in Industry Merit Award
1965-1968	Canadian National Research Council Special Studentship (overseas)
1968-1970	British National Research & Development Corporation Post-Doctoral Fellowship University College, London, England
1970-1971	Canadian Medical Research Council Post-Doctoral Fellowship McMaster University, Hamilton, Ontario, Canada
1978	Queen's Silver Jubilee Medal



1993 Distinguished Alumni Scholar Award, McMaster University

1997 Fellow, Royal Society of Canada

1998 Bernhard Cinader Award, The Canadian Society for Immunology

1998 Medal of Honour, Canadian Medical Association

1999 Who's Who in Healthcare Award

2002 Ortho Clinical Diagnostics Award, Ontario Society of Clinical Chemists

**APPOINTMENTS:**

1971-1973 Professional Assistant, Department of Medicine  
McMaster University, Hamilton, Ontario

1973-1975 Lecturer, Department of Pathology  
McMaster University, Hamilton, Ontario

1975-1978 Assistant Professor, Department of Pathology  
McMaster University, Hamilton, Ontario

1978-1984 Associate Professor, Department of Pathology  
McMaster University, Hamilton, Ontario

1976- Special Professional Staff  
Department of Laboratory Medicine  
McMaster University Medical Centre  
Hamilton, Ontario

1976- Director, Clinical Immunology Laboratory  
McMaster University Medical Centre  
Hamilton, Ontario

1984- Chief of Service of Immunology  
Department of Laboratory Medicine  
Chedoke-McMaster Hospitals, MUMC Division  
Hamilton, Ontario

1984- Professor, Department of Pathology  
McMaster University, Hamilton, Ontario

Jan - Sept 1988 Visiting Professor, Department of Immunology  
Scripps Clinic, La Jolla, CA

1989- Chairman, Department of Pathology and Molecular Medicine  
McMaster University, Hamilton, Ontario

1995-1998	Chief of the Department of Laboratory Medicine Chedoke-McMaster Hospitals, Hamilton, Ontario
1997-	Associate Member, Department of Biochemistry McMaster University, Hamilton, Ontario
1998-	Special Professional Staff, Department of Laboratory Medicine St. Joseph's Hospital
1999-	University Professor, Department of Pathology and Molecular Medicine McMaster University, Hamilton, Ontario
July 1999 - January 2000	Visiting Professor, The University of Edinburgh Edinburgh, Scotland
Jan 1, 2000 -June 30, 2005	John Bienenstock Chair in Molecular Medicine



### PROFESSIONAL ORGANIZATIONS:

American Association for Cancer Research  
 The American Association for Clinical Chemistry  
 American Association of Immunologists  
 The American Society for Investigative Pathology  
 American Thoracic Society  
 Canadian Chairs of Pathology and Laboratory Medicine  
 The Canadian Society of Clinical Chemists  
 The Canadian Society for Immunology  
 Canadian Thoracic Society  
 European Respiratory Society  
 International Cytokine Society  
 The Royal Society of Canada  
 United States and Canadian Academy of Pathology  
 Olympic Club of Canada (Munich 1972)

### SCHOLARLY AND PROFESSIONAL ACTIVITIES

#### Editorial Board:

American Journal of Physiology: Lung Cellular and Molecular Physiology  
 American Journal of Respiratory Cell and Molecular Biology  
 Cytokine  
 International Journal of Biochemistry and Cell Biology  
 Journal of Clinical Investigation  
 Journal of Immunology  
 Journal of Interferon and Cytokine Research

Scientific Advisory Board:

Rhone Poulenc Rorer, USA	1995-1998
Astra Draco, Sweden	1997-
Neurochem, Canada	1997-

Scientific Advisory Committees

MRC Centre for Inflammation Research at the University of Edinburgh	2001-
Norman Salvesen Emphysema Research Trust, University of Edinburgh	2001-
CIHR Institute of Infection and Immunity	
Institute Advisory Board Member	2001-
Strategic and Scientific Advisory Board (SSAB) of BioOntario	2002-

DEC 09 2002



Grant Committees:

The Arthritis Society  
 - Research Advisory Committee  
 1996-1999 Member

Canadian Institutes for Health Research (CIHR)  
 - Groups Peer Review Committee  
 2001- Member  
 - Canada Research Chairs College of Reviewers  
 2000 - Member

Francis Families Foundation  
 - Council of Scientific Advisors  
 1994- 1998 Member

Medical Research Council of Canada  
 - Grants Committee for Immunology and Transplantation  
 1990-1994 Member  
 1991-1994 Chairman  
 - Peer Review Restructuring Committee  
 1995 Member

Searle  
 - Arthritis and Prostaglandins Research Challenge Review Committee  
 1991-1993 Member

Journal Referee

American Journal of Pathology  
 American Journal of Respiratory Cell & Molecular Biology  
 American Journal of Respiratory and Critical Care Medicine  
 Arthritis and Rheumatism  
 Canadian Respiratory Journal  
 Gene Therapy  
 Infection and Immunity

International Journal of Biochemistry & Cell Biology  
 The Journal of Allergy and Clinical Immunology  
 The Journal of Clinical Investigation  
 The Journal of Immunology  
 Journal of Interferon and Cytokine Research  
 Journal of Leukocyte Biology



### External Grant Reviews

Alberta Lung Association  
 The Arthritis Society  
 British Lung Foundation  
 B.C. Health Research Foundation  
 Canadian Institutes of Health Research  
 NSERC  
 North Carolina Biotechnology Center  
 Wellcome Trust

### COURSES TAUGHT (last five years)

#### Undergraduate

B.Sc.N. Program Medical Microbiology Unit III	1994-
M.D. Immunology Large Group Session Unit 2	1990-
BHSc – Health Sciences 3J03	2002-

#### Graduate

MS723 Biochemistry	1998-
MS703 Gene Therapy	2001-

### SUPERVISORSHIPS

#### Masters

D. Williams	1979-1981
C. Richards	1983-1984
J. Wakeham	1997-1999
T. Galt	1997-2001
C. Thomson	1998-2001
M. Panju	1999-2001
S. Baral	1999-2001
V. Nethercot	1999-2001
G. Harder	2000-2002
L. Patton	2000-2002
M. Kelly	2000-
E. Safroneeva	2001-
S. Takenaka	2002-

Doctoral

L. Lamontagne	1977-1982
T. Egwang	1980-1984
A. Stadnyk	1983-1987
C. Richards	1984-1987
M. Jordana	1984-1989
Z. Xing, MD	1988-1994
M. Geisterfer	1988-1995
D. Torry	1988-1995
T. Braciak	1989-1996
K. Palmer	1994-2001
P. Emtage	1995-1998
S. Gyorffy	1996-1999
Q. Zhu	1999-
P. Margetts, MD	1999-
J. Robertson	2002-

Post-doctoral

L. Irving, MD	1985-1987
C. Vancheri, MD	1986-1988
C. Bradley, MD	1987-1989
A. Xaubet, MD	1988-1989
G. Cox, MD	1988-1990
I. Ohno, MD	1990-1992
J. Sallenave, PhD	1992-1995
G. Tremblay, PhD	1993-1995
P. Sime, MD	1993-1998
R. Foley, MD	1993-1997
L. Waldhauser, PhD	1994-1998
J. Bramson, PhD	1994-1997
Y. Wan, PhD	1995-1998
A. Redington, MD	1996-1997
K. Takahashi, PhD	1996-1997
P. Jones, PhD	1998-2000
Y. Chen, MD	1998-2001
J. Tao, MD, PhD	1998-1999
M. Kolb, MD	1999-
C. Dabrosin, MD, PhD	1999-2001
S. Gyorffy, PhD	2000-2001
P. Bonniaud, MD	2001-
J.C. Rodriguez, DVM, PhD	2002-

Supervisory Committees

R. Slapsys, PhD	1981-1986
J. Ramwani, PhD	1982-1986
J. Rudolph, PhD	1982-1989

A. Faggioto, PhD	1983-1985
D. Gasangwa, MSc	1985-1987
S. Polyak, PhD	1988-1993
A. Agro, PhD	1988-1996
R. Quezuda-Calvillo, PhD	1989-1994
K. Woolley, PhD	1990-1993
C. Addison, PhD	1992-1997
G. Gauvreau, PhD	1993-1998
B. Cowan, PhD	1994-1998
S. Ruiz, PhD	1994-1996
N. Radojevic, MSc	1996-1998
P. Heritage, PhD	1997-1999
P. Koeberle, PhD	1997-
B. Gajewska, PhD	1997-
K. Sussman, MSc	1997-
A. Kashyap, MSc	1997-2001
S. Ritz, PhD	1998-
M. Rosenblatt, MSc	1999-2001
R. Leigh, PhD	2000-
M. Landis, MSc	2001-
J. Bezhlibnyk, PhD	2001-
F. Swirski, PhD	2001-
Y. Trieu, MSc	2001-
R. Wiley, PhD	2001-
A. Kwant, MSc	2002-
D. Smyth, MSc	2002-
P. Hew, MSc	2002-
R. Fattouh, MSc	2002-
D. Smyth, MSc	2002-

## ADMINISTRATIVE RESPONSIBILITIES

### International:

IUIS Clinical Immunology Committee  
 1991- CSI Representative

Keystone Symposia - "The Cellular and Molecular Regulation of the Acute Inflammatory Response"  
 February 7-12, 1994, Durango, Colorado  
 Co-organizer

2nd International Cytokine Conference  
 October 1-5, 1994, Banff, Alberta  
 Organizing Committee

American Thoracic Society - Assembly in Allergy, Immunology & Inflammation  
 1994-1998 - Program Committee Member  
 1994-1997 - Long-Range Planning Committee Member

**National:**

Arthritis Society of Canada  
1996- Member, Research Advisory Committee

Association of Canadian Medical Schools and Colleges  
1980-1982 Chairman, M.D. Admissions Officers Subcommittee

Canada Research Chairs  
2000- College of Reviewers

Canadian Society for Immunology  
1993-1997 Council Member

Canadian Society for Laboratory Technologists  
1976-1979 Clinical Immunology Chief Examiner

Medical Research Council of Canada  
1993-1994 Advisory Committee for Peer Review

National Research Council Canada  
1993-1997 Advisory Board - Institute for Biological Sciences (IBS)

**Local:**

The Regional Municipality of Hamilton-Wentworth  
The Renaissance Project

1993 - 1995 Member

**Hamilton Health Sciences Laboratory Program****Academic Advisory Committee**

1976- Member  
1989- Chairman

**Operational Management Committee**

1989- Member  
1995-1998 Chairman

**Coordinating Committee**

1989- Member

**Faculty of Health Sciences**

School of Medicine  
1978-1982 Chairman, M.D. Admissions Committee

Committee on Scientific Development  
1981-1982 Member

## 1982-1985 Executive

## Striking Committee

1981-1982 Member  
1982-1985 Chairman

## Board of Comprehensive Examiners

1981-1983 Member  
1983-1986 Chairman

## Dean's Executive 1989-1990

## Faculty Executive 1989-

## Council of the Faculty of Health Sciences 1989-

## **Clinical Chairmen Committee 1989-**

## Health Services Advisory Committee Executive 1989-1993

## Ad Hoc Advisory Group - Institute for Molecular Biology & Biotechnology 1989-

## Faculty Finance Committee 1990-1994

## Primate Research Review Committee 1990-1991

## Rheumatology Task Force 1990-1993

## Life Sciences Council 1991-

## Implementation Coordinating Committee 1993-1994

## Task Force on Molecular and Physiological Sciences 1993-1994 Chair

# McMaster/Mohawk Joint Initiative Concept Team 1993-1994

RESEARCH FUNDING (last five years)

AstraZeneca

“Airways tissue remodelling”

Jack Gauldie

January 1 – December 31, 1998

\$134,400 pa

January 1 – December 31, 1999

\$134,400 pa

January 1 – December 31, 2000

\$115,500 pa

Baxter Healthcare Corp.

“A model of peritoneal fibrosis”

Jack Gauldie

December 15, 1998 – December 14, 1999

\$123,250 pa

November 30, 1999 – November 30, 2000

\$133,000 pa

Breast Cancer Society of Canada

“Dendritic cell and gene-based therapy of breast cancer using HER-2/neu antigen”

Yonghong Wan, Jack Gauldie

July 1, 1999 – June 30, 2000

\$ 25,000 pa

July 1, 2000 – June 30, 2001

\$ 25,000 pa

Canadian Institutes of Health Research

“Cytokine gene transfer modulation of mucosal immunity”

Jack Gauldie

April 1, 1999 – March 31, 2004

\$146,256 pa

“The pathogenesis of pulmonary fibrosis”

Jack Gauldie

October 1, 1997 - September 30, 2002

\$ 88,975 pa

“Genetic immunotherapy of cancer”

A. Keith Stewart, Jack Gauldie, Frank L. Graham, Mary Hitt, John Trachtenberg

\$103,000 pa

October 1, 2001 – September 30, 2006

\$168,700 pa

“Phase II Study, Multiple injections of autologous CD34<sup>+</sup>”

Ronan Roley, Jack Gauldie, Mark Levine, Dave Tozer

April 1, 2000 – March 31, 2002

\$ 82,100 pa

“TH2 cytokine gene transfer in regulation of inflammation and immunity”

Jack Gauldie

July 1, 1994 - March 31, 1999

\$ 83,916 pa

Geron Corporation

“Development of telomerase-based cancer vaccine”

Jack Gauldie

September 1, 2000 – August 31, 2001

\$ 97,440

Glaxo



"Adenovirus vector-mediated cytokine gene transfer to the lung"

Jack Gauldie

June 30, 1997 – June 30, 1998

\$137,850 pa

HHSC Foundation Sloat Fund

Ronan Foley, Peter Dent, Jack Gauldie, Peter McCulloch, Ralph Meyer,  
J. Rusthoven, R. Tozer, Yonghong Wan

1998

\$ 58,860 pa

1999

\$ 58,860 pa

Leukemia Research Fund of Canada

"Gene therapy of CLL"

Ronan Foley, Jack Gauldie, Yonghong Wan

July 1, 1998 – June 30, 2000

\$ 25,000 pa

NCE – CANVAC

CANVAC 3.2.4 "Adenovector modified dendritic cells expressing tumor antigen (HER)"

Jack Gauldie

April 1, 2000 – March 31, 2007

\$ 90,000 pa

CANVAC 3.4.2 "Tumor antigen delivery via adenovirus modified dendritic cells – clinical studies"

Jack Gauldie

April 1, 2000 – March 31, 2007

\$115,000 pa

CANVAC CORE – "Models for testing delivery systems"

Jack Gauldie

April 1, 2000 – March 31, 2007

\$ 50,000 pa

CANVAC 3.2.3 – "Modifying adenovectors to allow sustained antigen delivery (SEROAD)"

Jack Gauldie and Frank Graham

April 1, 2000 – March 31, 2007

\$110,000 pa

NIH

Subcontract Agreement for Grant #PO1 HL60231-01 with Children's Hospital of Los Angeles  
"Molecular basics of lung morphogenesis, injury and repair"

Jack Gauldie

April 1, 1998 – March 31, 2003

US \$ 41,376 pa

Ontario Thoracic Society

"Modifications of intranasal immunity using cytokine expressing adenoviruses"

Mark McDermott, Jack Gauldie

July 1, 1998 – June 30, 1999

\$ 23,250 pa

Roche (Boehringer-Mannheim GmbH)

"Research program for the construction and characterization of CDA vector"

Jack Gauldie, Frank Graham

July 1, 1998 – June 30, 2000

\$250,800 pa

July 1, 2000 – June 30, 2001

\$125,400 pa

February 1, 2001 – January 31, 2002

\$ 80,080 pa



**PATENTS****United States Patent**

Patent Number: 4,973,478  
 Date of Patent: November 27, 1990  
 Inventors: Jack Gauldie, Carl Richards and Peter M. Lansdorp  
 Title: Treating inflammation with hepatocyte stimulating factor interferon E2

**United States Patent**

Patent Number: 7,935,097  
 Date Filed: August 26, 1992  
 Inventors: Carl Richards, Mohammed Shoyab, Jack Gauldie and Tom Brown  
 Title: Regulation of cellular invasiveness

**United States Patent**

Patent Number: 8,250,885  
 Date Filed: May 31, 1994  
 Inventors: Frank Graham, Jack Gauldie, William Muller and Christina Addison  
 Title: Direct intratumoral injection of recombinant adenovirus vectors and viral particles that encode cytokines, to obtain shrinkage and elimination of tumors.  
 Description: The patent describes the use of adenovirus vectors expressing cytokines for immunotherapy of cancer. Vectors have been shown to cause the shrinkage and total regression of tumors in a transgenic murine model system for breast cancer following direct intratumoral injection.

**United States Patent Pending**

Patent Number: 09/360,199  
 Inventors: Bruce A. Vallance, Stephen M. Collins, Jack Gauldie, Yonghong Wan  
 Title: Intestinal gene therapy

**United States Patent Pending**

Patent Number: Filed September 25, 1999  
 Inventors: Yonghong Wan, William J. Muller, Jack Gauldie, Jonathan Bramson  
 Title: Cancer immunotherapy targeting ErbB-3

**United States Patent Pending**

Patent Number: Filed September 25, 1999  
 Inventors: Yonghong Wan, William J. Muller, Jack Gauldie, Niki Sharan, Kay Palmer, Peter Emtage  
 Title: Application of modified receptor tyrosine kinases for cancer gene therapy

**United States Patent Pending**

Patent Number: Filed December 13, 1999  
 Inventors: Yonghong Wan, Jack Gauldie, Jonathan Bramson  
 Title: Blockade of T cell activation pathway for inducing auto-immunity

**United States Patent Pending**

Patent Number: Filed April 26, 2000

Inventors: Yonghong Wan, Jonathan Bramson, Jack Gauldie

Title: Methods of modulating the immune system response to self-antigens

**United States Patent Pending**

Patent Number: 09/742,892 Filed 12/21/2000

Inventors: Todd Braciak, Vipin Kumar, Eli Sercarz, Jack Gauldie, Peter Emtage, Frank Graham

Title: Recombinant genetic vaccine for the prevention and treatment of acne

Papers Published or In Press in Refereed Journals

1. Vernon, C.A., Gauldie, J., Hanson, J.M., Humphreys, J.M., Smith, P.E., Lawrence, A.J. and Banks, B.E.C. Acid Phosphatases. *Nature* 208:382-383, 1965.
2. Banks, B.E.C., Doonan, S., Gauldie, J., Lawrence, A.J. and Vernon, C.A. The dissociation into subunits of aspartate aminotransferase from pig-heart muscle. *Eur. J. Biochem.* 6:507-513, 1968.
3. Hillcoat, B.L., Marshall, L., Gauldie, J. and Hiebert, M. Stabilization of dihydrofolate reductase by inhibitors *in vivo* and *in vitro*. *Ann. NY Acad. Sci.* 186:187-208, 1971.
4. Hiebert, M., Gauldie, J. and Hillcoat, B.L. Multiple enzyme forms from protein-bromophenol blue interaction during gel electrophoresis. *Anal. Biochem.* 46:433-437, 1972.
5. Gauldie, J. and Hillcoat, B.L. Purification of tetrahydrofolate dehydrogenase by affinity chromatography. *Biochim. Biophys. Acta* 268:35-40, 1972.
6. Bienenstock, J., Perey, D.Y.E., Gauldie, J. and Underdown, B.J. Chicken immunoglobulin resembling gamma A. *J. Immunol.* 109:403-406, 1972.
7. Gauldie, J., Marshall, L. and Hillcoat, B.L. Purification and properties of dihydrofolate reductase from cultured mammalian cells. *Biochem. J.* 133:349-356, 1973.
8. Bienenstock, J., Perey, D.Y.E., Gauldie, J. and Underdown, B.J. Chicken gamma A: Physiochemical and immunochemical characteristics. *J. Immunol.* 110:524-533, 1973.
9. Dolovich, J., Hargreave, F.E., Chalmers, R., Shier, K.J., Gauldie, J. and Bienenstock, J. Late cutaneous allergic responses in isolated IgE-dependent reactions. *J. Allergy Clin. Immunol.* 52:38-46, 1973.
10. Mant, M.J., Hirsh, J., Gauldie, J., Bienenstock, J., Pineo, G.F. and Luke, K.H. Von Willebrand's syndrome presenting as an acquired bleeding disorder in association with a monoclonal gammopathy. *Blood* 42:429-436, 1973.
11. Bienenstock, J., Gauldie, J. and Perey, D.Y.E. Synthesis of IgG, IgA, IgM by chicken tissues: Immunofluorescent and <sup>14</sup>C amino acid incorporation studies. *J. Immunol.* 111:1112-1118, 1973.
12. Gauldie, J., Bhandari, S.C. and Singal, D.P. Alteration of the HL-A antigenic site *in situ*. *Immunol. Commun.* 4:465-476, 1975.
13. Mant, M.J., Doery, J.C.G., Gauldie, J. and Sims, H. Pseudothrombocytopenia due to platelet aggregation and degranulation in blood collected in EDTA. *Scand. J. Haematol.* 15:161-171, 1975.
14. Clancy, R.L., Gauldie, J., Vallieres, M., Bienenstock, J., Day, R.P. and Pineo, G.F. An approach to immunotherapy using antibody to IgE in mast cell leukemia. *Cancer* 37:693-696, 1976.

15. Keane, P.M., Walker, W.H.C., Gauldie, J. and Abraham, G.E. Thermodynamic aspects of some radioassays. *Clin. Chem.* 22:70-73, 1976.
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Exhibit B: Adenoviral-based gene delivery in the  
lower GI tract induces antigen-specific immune  
responses and protection from Tumour challenge





## **Adenoviral-based gene delivery in the lower GI tract induces antigen-specific immune responses and protection from Tumour challenge**

### **Background:**

The entry of pathogenic organisms most often occurs at the mucosal surfaces. To prevent infectious diseases, such as sexually transmitted disease, in the genital-urinary (GU) and the lower gastrointestinal (GI) tracts, induction of protective immunity at these mucosal sites against microbial access is critical. Induction of systemic immune responses against further invasion of pathogens into the body through the mucosa is also important. In addition, induction of a potent Cytotoxic T Lymphocyte response (CTL) is important both for control of viral infections and for tumour surveillance and protection.

### **Aim:**

To investigate potential of local gene expression within the rectal tissue, using adenoviral vectors (AdV), to promote local mucosal and systemic antigen-specific cell and humoral immune responses. To document the efficacy of the rectal route for generation of protective immunity at both the local tissue site and for systemic immunity.

### **Methods:**

1. AdV encoding the LacZ reporter gene (AdLacZ) or an immunogenic antigen, such as chicken ovalbumin (AdOVA  $5 \times 10^9$  pfu), was administered intrarectally (iR) through the anus of mice. Mice were first subjected to a 50% Ethanol wash (enema) for one hour. AdV was then delivered into the lumen of the colo-rectum.
2. 14 days post iR immunization with AdOVA, a homologous tumor cell line (EL4) expressing OVA antigen (E.G7-OVA) was injected intra-mucosally in the rectal tissue (local challenge) or subcutaneously (systemic challenge) into mice and tumor formation and growth was followed

over a period of time at regular intervals as well as survival on the mouse. When tumor volumes reached more than 1000 mm<sup>3</sup>, mice were euthanized.

3. Colo-rectal tissues and local draining lymph nodes (iliac node) as well as non-draining nodes (cecal node) were examined for gene expression and local responses to the OVA gene. 14 days after AdOVA administration, spleen cells were also collected for measurement of cytokine production and determination of antigen-specific CTL activities using specific OVA peptide (SIINFEKL) pulsed target cells (EL4 based). Mice deficient in CD8 T cells (CD8 KO) were used to investigate the role of CD8 T cells in the CTL response and protection from tumour challenge.

### **Results:**

1. Staining for  $\beta$ -galactosidase showed that the expression of the transferred gene was widespread across the crypts and villi of the colo-rectal epithelial layer from one to three days post iR administration (Fig 1, 4, 5). The transgene expression was dose dependent (Fig 6).
2. 14 days after AdOVA iR immunization, mice were protected from tumor challenge with OVA-antigen expressing tumour cells (EG7-OVA) either delivered to a systemic site, subcutaneously (Fig 2, 9, 10), or to a local site, intra-mucosally (Fig 11). The protection from tumour challenge after iR immunization was shown to be CD8 dependent through the use of CD8 KO mice where the protection was abolished (Fig 12). Protection was not seen when OVA protein alone was delivered to the rectal tissue (Fig 12).
3. OVA-specific systemic (spleen) and local (Draining lymph nodes) CTL responses were detected 14 days after AdOVA iR immunization (from 20% to 60% in separate experiments vs. ~1% in the control) (Fig 3, 7, 8).
4. The production of INF- $\gamma$ , but not IL-4, was dramatically increased in the culture of spleen cells re-stimulated with OVA protein (1076.7 pg/ml vs. 42.8 pg/ml).

**Conclusions:**

1. Transgenes can be effectively delivered by AdV in the lower GI tract and expressed widespread across the crypts and villi of the colo-rectal mucosal surfaces.
2. Adenovirus-based mucosal intra-rectal (iR) gene delivery induces both strong systemic and local mucosal immune responses, which are antigen specific. This method offers direct advantages as a vaccination route to induce local immune responses within the colo-rectal tissue and within the common mucosal tissue in general. This route of immunization also offers induction of protective CTL activity and long-lasting immune protection from tumour challenge.

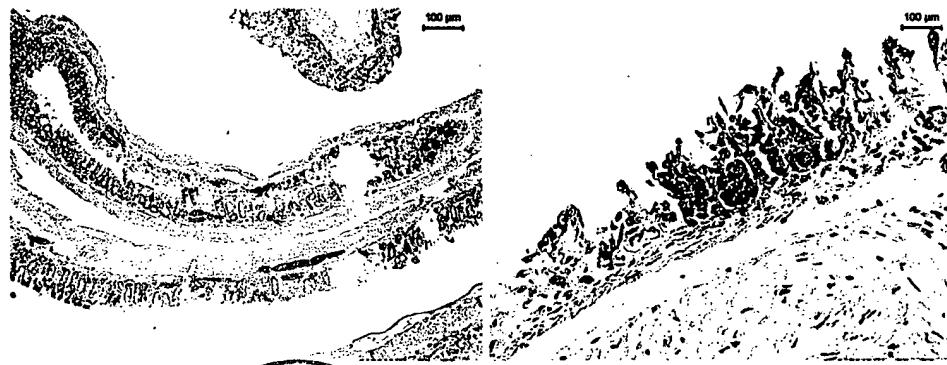
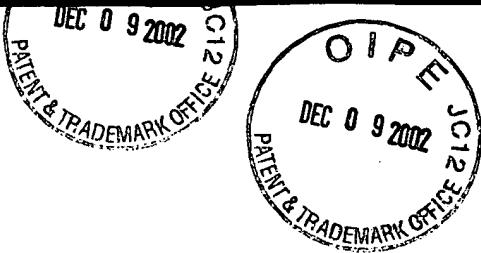


Fig 1. LacZ expression visualized in the colo-rectum one day after AdLacZ iR delivery.

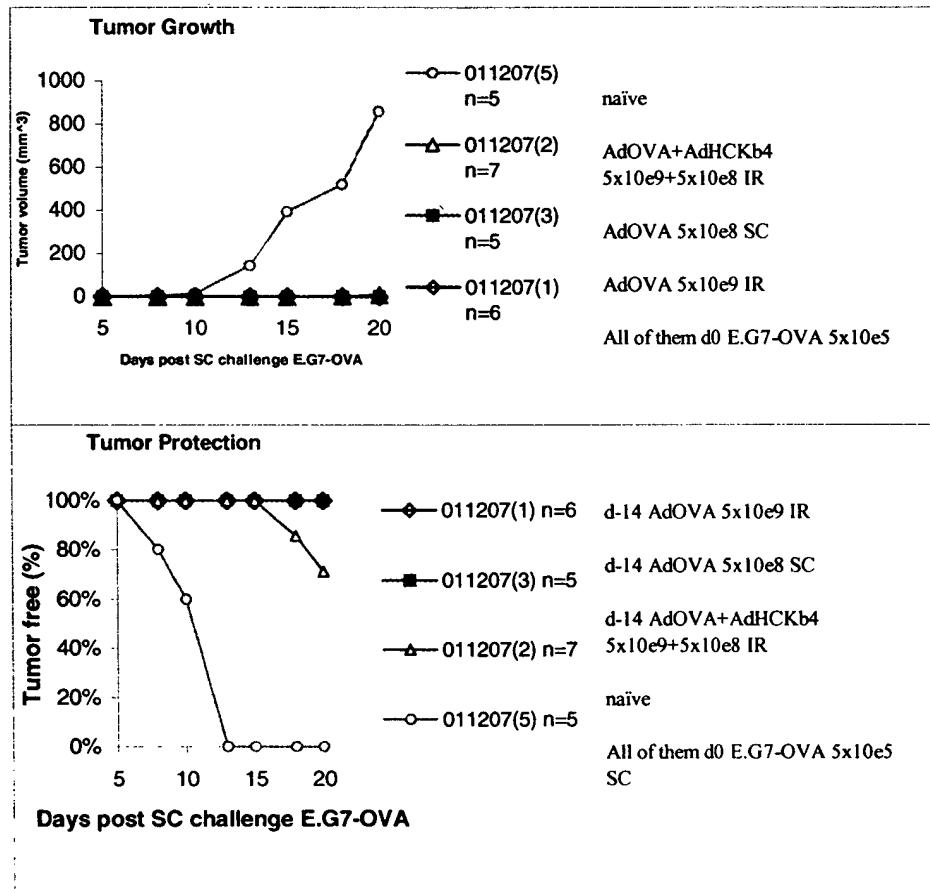


Fig 2. Tumor protection and tumor growth after E.G7-OVA challenge in iR AdOVA-immunized mice.

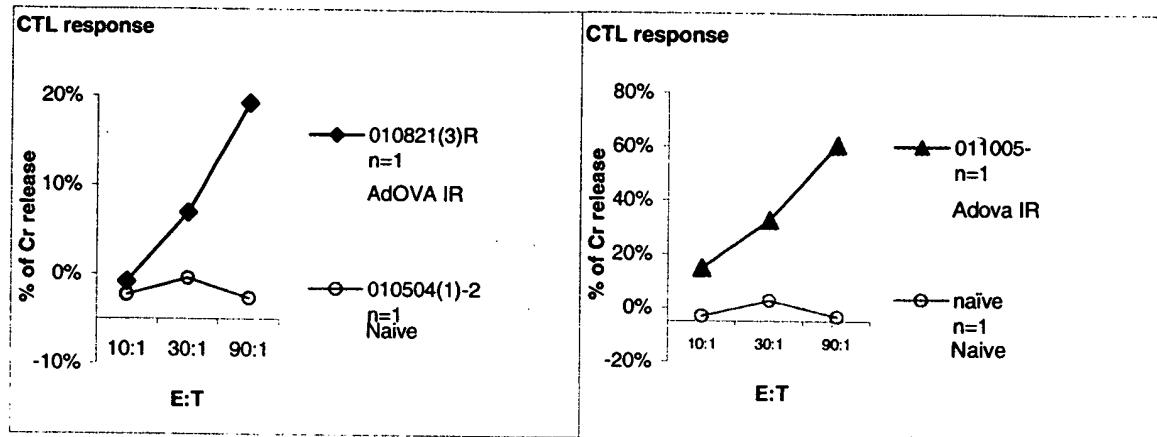


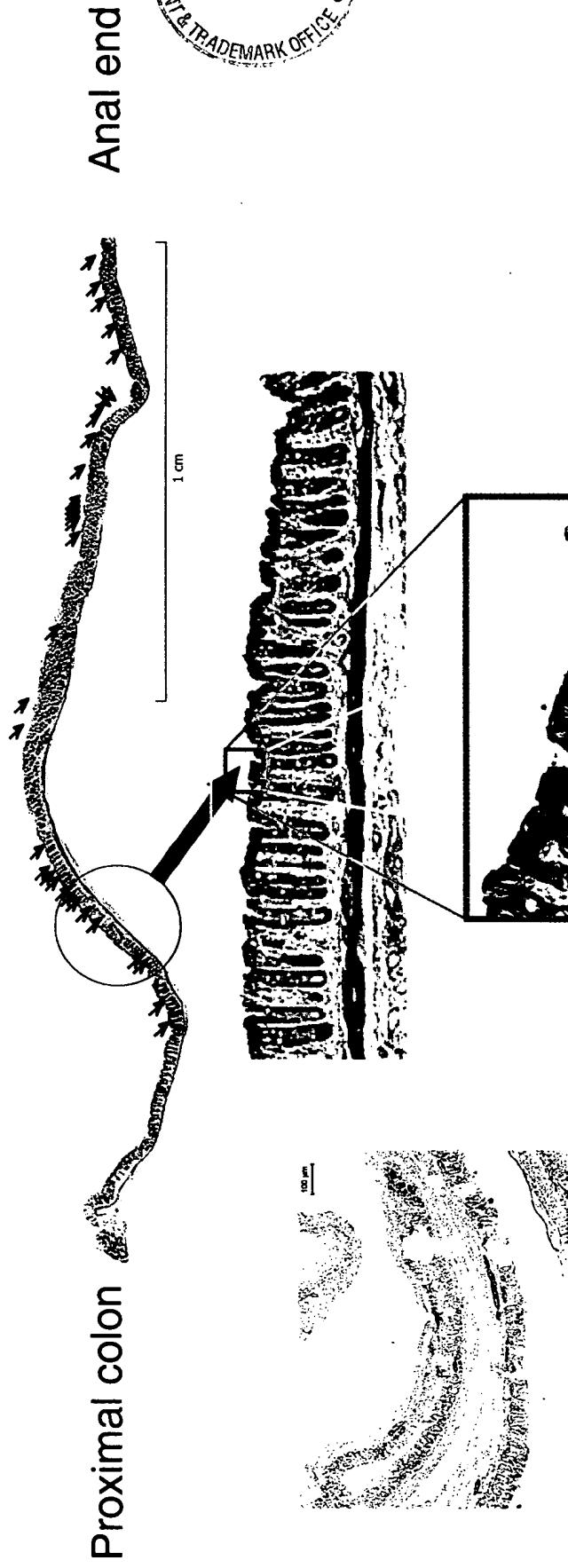
Fig 3. Spleen CTL response against EL-4 pulsed with OVA peptide SINFEKL. The results represent the CTL response from 2 independent experiments.



Visualization of gene expression in the colon

Day 2 after AdLacZ 5x10<sup>9</sup> pfu IR

Fig 4



Gene expression in the colo-rectum  
day 1 up to day 3.  
LacZ staining with X-gal





## Visualization of gene expression in the colon

Fig 5

Day 2 after AdLacZ 5x10<sup>9</sup> pfu IR



## Anti-LacZ Ab

## AdLacZ staining

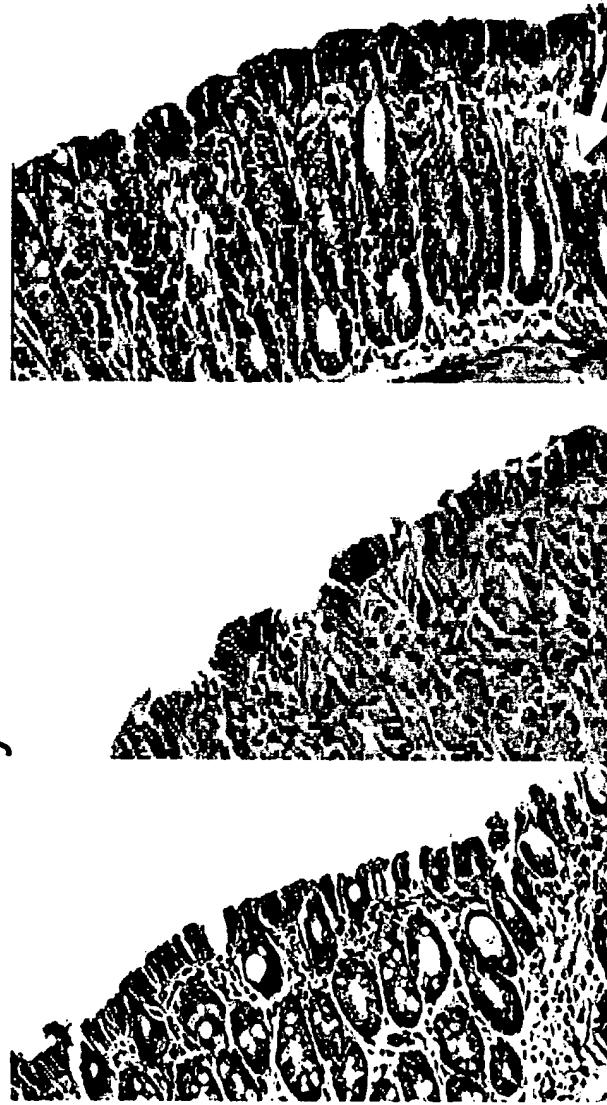
Gene expression is found across the villi and crypts of the epithelium and “maybe the LP” of the colon, implying efficiency of gene delivery by Adv at high doses.



## Visualization of gene expression in the colon

--- Dose dependent study -----

Day 3 after AdLacZ IR



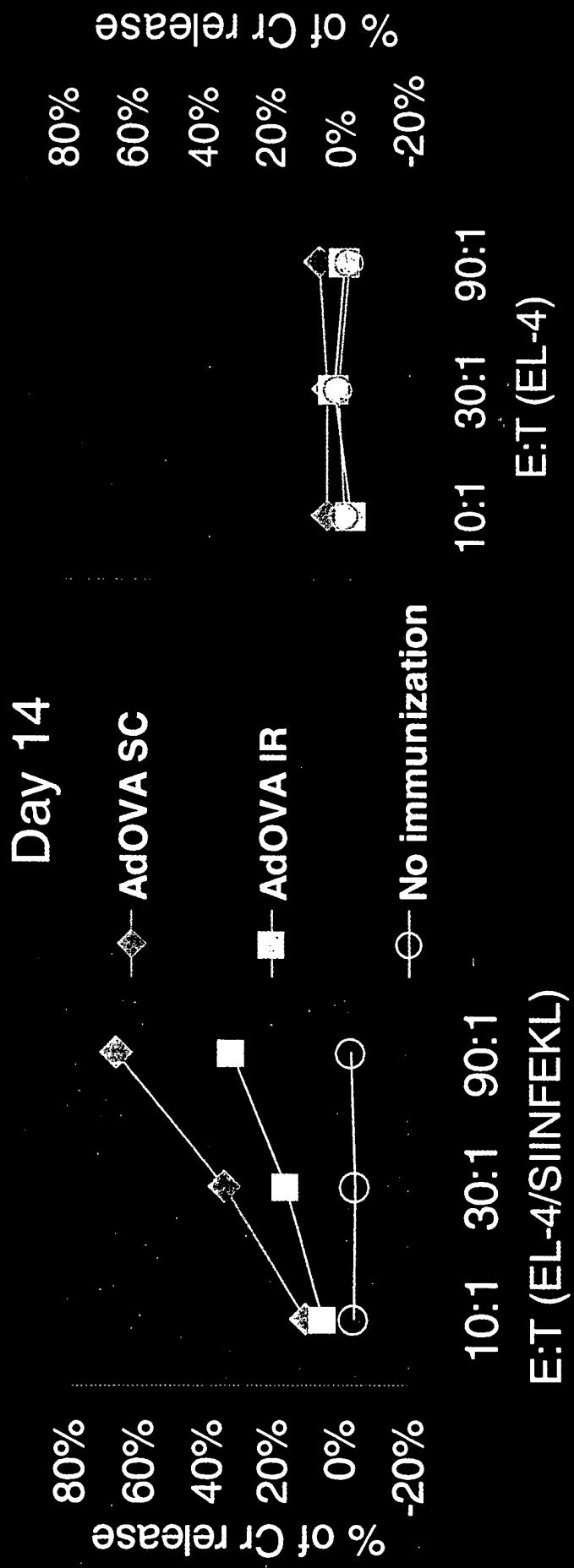
$5 \times 10^8$  pfu       $1 \times 10^9$  pfu       $2 \times 10^9$  pfu

Adv IIR-administrated gene can be delivered onto the epithelial cells and be expressed.

Fig 6



# Induction of cellular immune responses by AdOVA IR --- Systemic (spleen)---

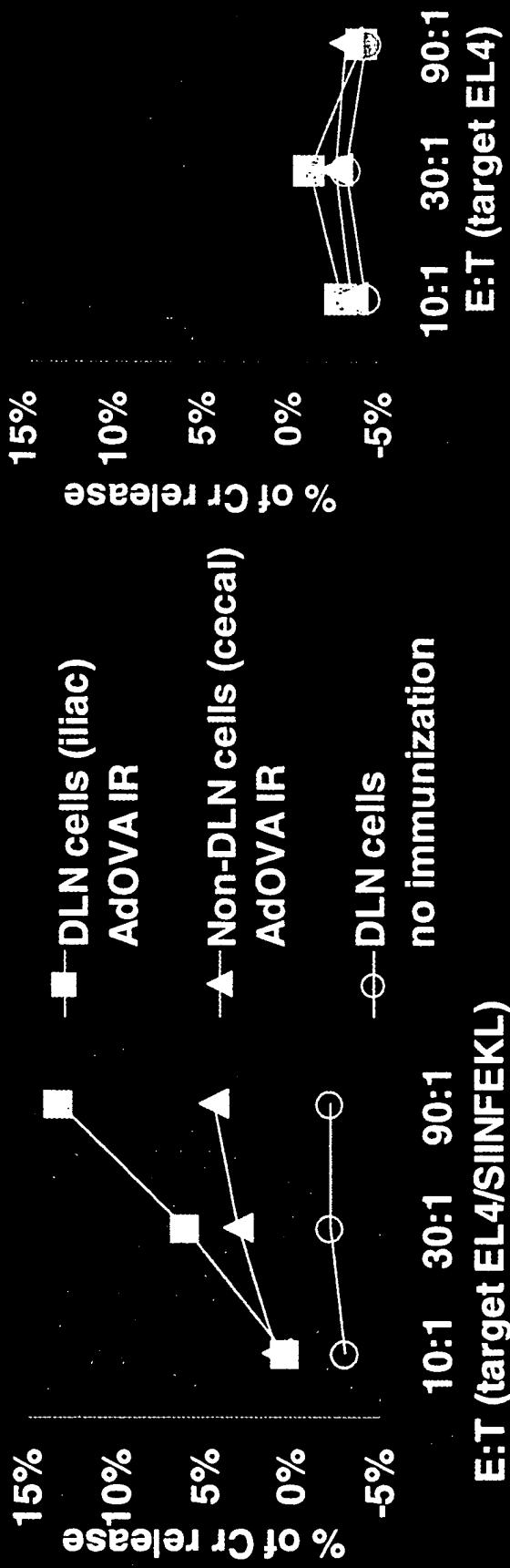


Systemic CTL responses induced by AdOVA IR were antigen-specific.

Fig 7

# Induction of cellular immune responses after AdOVA IR

--- Local (DLNs) ---  
Day 5



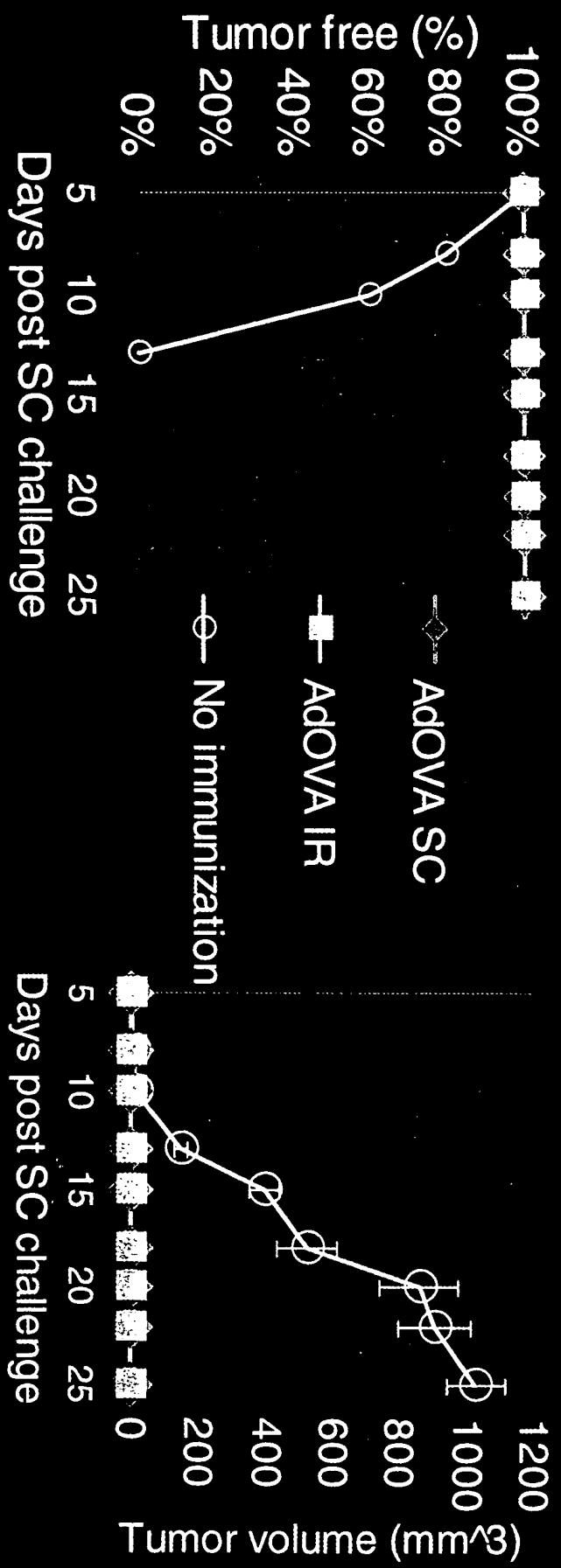
Local DLN primary CTL responses induced by AdOVA IR were also antigen-specific.

Fig 8

# Induction of protective immunity by AdOVA IR

--- Systemic immunity against tumor challenge ---

## Short term



Systemic immune responses induced by AdOVA were protective

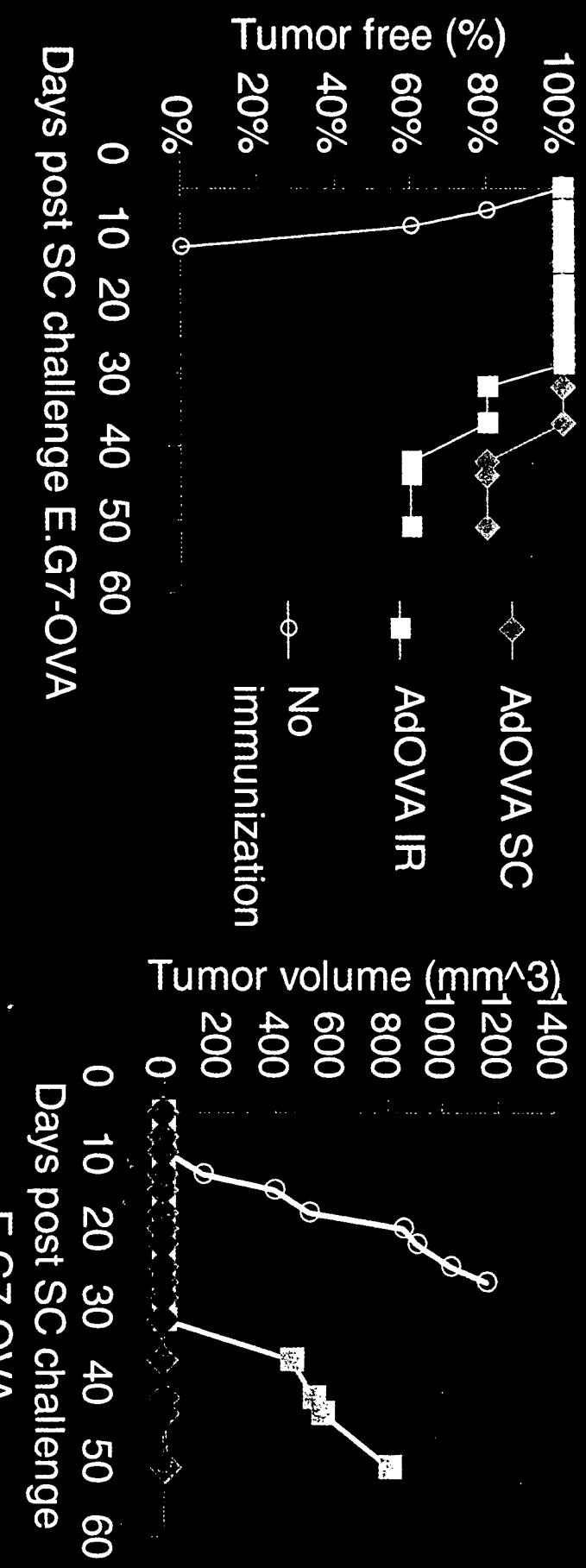
Fig 9



# Induction of protective immunity by AdOVA IR

--- Systemic immunity against tumor challenge ---

## Long term

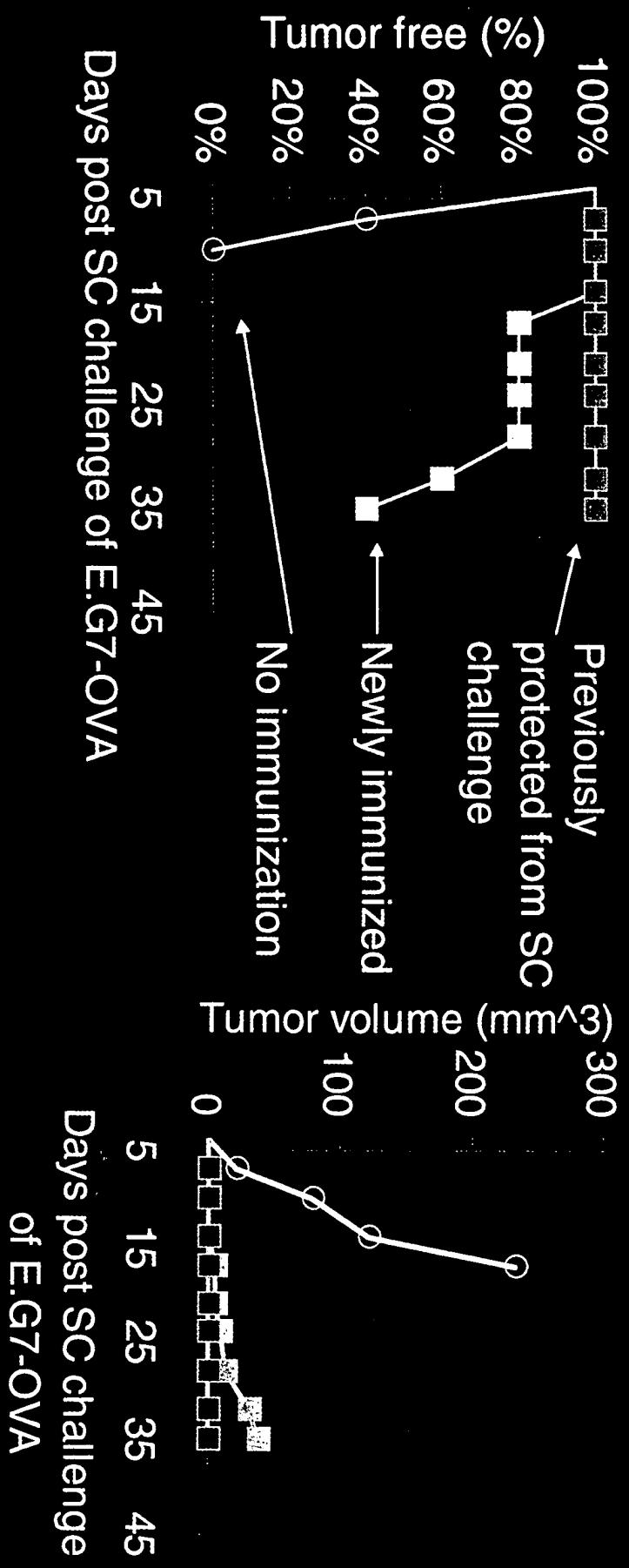


Systemic immune responses induced by AdOVA were protective

Fig 10



# Induction of protective immunity by AdOVA IR Local immunity against tumor challenge in the rectal mucosa



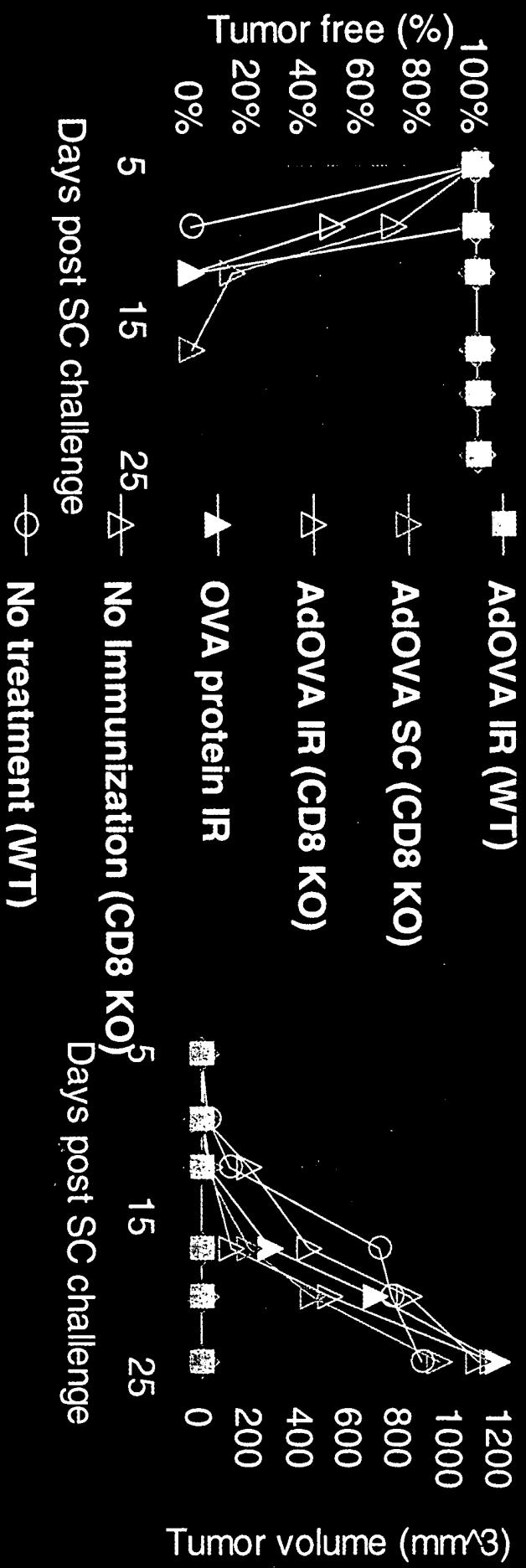
Mucosal immune responses induced by AdOVA were protective

Fig 11



# Induction of protective immunity by AdOVA IR

--- CD8 dependent ---



The CTL responses are CD8 dependent (CD8 KO not protected).  
Also, OVA protein alone was not able to induce protective immune responses

Fig 12

# Exhibit C: Induction of protective distal mucosal immunity against HSV-2 infection





## Summary: Induction of protective distal mucosal immunity against HSV-2 infection

### Abbreviations:

Adv	Adenoviral vector
IR	Intrarectal
IVAG	Intravaginal
gB	Glycoprotein B
pfu	Plaques forming unit
HSV-2	Herpes simplex virus type 2
tw	Tissue weight
LD <sub>50</sub>	50% lethal dose
vw	Vaginal wash

## Materials and Methods

### *Animals, cell cultures and viruses*

Female C57BL/6 mice were 6-8 weeks of age during immunization with Adv. Vero cells were grown in complete  $\alpha$ -MEM media. Recombinant AdgB is an Adenovirus vector that encodes gB8, the surface protein gene from HSV. HSV-2 strain 333 was propagated and titered on Vero cells.

### *IR immunization and virus challenge*

Mice were anesthetized with isoflurane and instilled with 50% ethanol into the colo-rectum, and kept under anesthesia for 30 min. One hour later, AdgB was IR delivered by insertion of a pipet tip into the rectum, followed by one-hour incubation period. 21 days after AdgB IR immunization, mice were IR (rectal challenge) or IVAG (vaginal challenge) challenged with HSV-2 strain 333. For IR challenge, the procedure was as the same as that for IR immunization. For IVAG challenge, mice were injected SC with 2.5 mg of progesterone (Depo-Provera) 5 days prior to administration of HSV-2. 20  $\mu$ l of HSV-2 was given IVAG, followed by one-hour incubation.

### *Viral replication and pathology in the anal and genital tract*

After IVAG inoculation of HSV-2, vaginal washes were obtained daily by pipetting twice 30  $\mu$ l of PBS into and out of vaginal tract, and stored at -70°C before use. Virus shedding was determined by plaque assay on Vero cell monolayers and expressed by virus retrieved from per vaginal wash (vw) in 60  $\mu$ l.

Anal or genital pathology was monitored and scored daily after HSV-2 challenge. Genital pathology was scored by a 6-point scale from 0 to 5 (adapted from Overall et al, 1975; Gallichan et al, 1998, 2001; Kuklin et al, 1998): 0, no change; 1, redness of external vagina; 2, swelling of external vagina, severe redness; 3, perineal hair loss or genital ulceration, severe swelling; 4, perineal ulceration; and 5, hind limb paralysis or death. Anal pathology was also scored on a 6-point scale based on the descriptions by Phillips et al (1998, 2000): 0, no change; 1, redness of anus; 2, swelling of anus, severe redness; 3, perineal hair loss or anal ulceration, severe



swelling of anus and perineum; 4, perineal lesion; and 5, hind limb paralysis, anal restrictions or death.

## Results

### *Dose dependent study of IR challenge of HSV-2*

To determine the LD<sub>50</sub>, naive mice were IR inoculated with HSV-2 strain 333, and monitored for anal pathology (daily for the first 2 weeks). As shown in Table 1, 50% of mice died from HSV-2 IR inoculation at a dose of 2×10<sup>4</sup> pfu. Anal pathology developed rapidly and no mice survived when the doses were increased to 2×10<sup>5</sup> pfu and 2×10<sup>6</sup> pfu. When mice received the latter dose, which is 100-fold higher than LD<sub>50</sub>, they were all paralyzed by the first week. Because this dose was highly lethal, leading to early onset of anal pathology and rapid death, it was used to challenge AdgB IR-immunized mice.

Table 1. Survival rate (%) of naive mice IR inoculated with HSV-2 (8 mice/group).

Dose (pfu)\week	1	2	3	4
2×10 <sup>3</sup>	100	75	75	75
2×10 <sup>4</sup>	87.5	50	50	50
2×10 <sup>5</sup>	87.5	0	0	0
2×10 <sup>6</sup>	0	0	0	0

### *Pathology and survival from IntraRectal HSV-2 challenge in AdgB IR-immunized mice*

21 days after a single IR immunization with AdgB, mice were monitored for pathology and survival following a lethal IR challenge of 2×10<sup>6</sup> pfu of HSV-2. All unimmunized mice (n=8) rapidly developed pathology, and were unable to survive the challenge by day 7. In AdgB IR-immunized mice (n=12), 41% of mice had overt pathology and 92% survived the HSV challenge. For those mice that developed pathology, the severity of infection was less than non-immunized mice (maximum score points: 3.6±0.9 vs. 5±0.0), and external indications of infection were no longer visible by week 2, indicating the ability of immunized mice to withstand the infection at high lethal doses.

The development of genital pathology after challenge of 2×10<sup>5</sup> pfu of HSV-2, which is 10-fold higher than conventional dose (2×10<sup>4</sup> pfu), was also assessed. All unimmunized mice died within the first week of challenge, whereas 100% of immunized mice survived. Although 60% of immunized mice demonstrated overt genital pathology (3.7±0.6 vs. 5±0.0), they were also able to control the infection, characterized by regression of some mild perineal lesions.

*Virus titers in vaginal washes of IntraVaginal HSV-2 challenge in AgB IR immunized mice*



Previous studies have shown that virus shedding peaks at day 3 post infection. Virus shedding was compared on monolayer Vero cells by measuring plaques formed by virus obtained from vaginal washes. Three days after HSV challenge at a dose of  $2 \times 10^5$  pfu, virus was detected in the samples of all unimmunized mice ( $8.0 \times 10^3 \pm 3.7 \times 10^3$  pfu/vw, n=5). Although 80% of immunized mice (n=5) were detected positive for virus shedding, compared to those from unimmunized mice, the virus titers from these immunized mice were at least one log lower ( $8.7 \times 10^2 \pm 7.0 \times 10^2$  pfu/vw). While all unimmunized mice retained similar levels of virus shedding until death, 40% of immunized mice were no longer positive for virus by day 5, and all were virus free by day 10.

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**Adenoviral-based gene delivery in the lower GI tract induces antigen-specific immune responses and protection from Tumour challenge**

**Background:**

The entry of pathogenic organisms most often occurs at the mucosal surfaces. To prevent infectious diseases, such as sexually transmitted disease, in the genital-urinary (GU) and the lower gastrointestinal (GI) tracts, induction of protective immunity at these mucosal sites against microbial access is critical. Induction of systemic immune responses against further invasion of pathogens into the body through the mucosa is also important. In addition, induction of a potent Cytotoxic T Lymphocyte response (CTL) is important both for control of viral infections and for tumour surveillance and protection.

**Aim:**

To investigate potential of local gene expression within the rectal tissue, using adenoviral vectors (AdV), to promote local mucosal and systemic antigen-specific cell and humoral immune responses. To document the efficacy of the rectal route for generation of protective immunity at both the local tissue site and for systemic immunity.

**Methods:**

1. AdV encoding the LacZ reporter gene (AdLacZ) or an immunogenic antigen, such as chicken ovalbumin (AdOVA  $5 \times 10^9$  pfu), was administered intrarectally (iR) through the anus of mice. Mice were first subjected to a 50% Ethanol wash (enema) for one hour. AdV was then delivered into the lumen of the colo-rectum.
2. 14 days post iR immunization with AdOVA, a homologous tumor cell line (EL4) expressing OVA antigen (E.G7-OVA) was injected intra-mucosally in the rectal tissue (local challenge) or subcutaneously (systemic challenge) into mice and tumor formation and growth was followed

over a period of time at regular intervals as well as survival on the mouse. When tumor volumes reached more than 1000 mm<sup>3</sup>, mice were euthanized.

3. Colo-rectal tissues and local draining lymph nodes (iliac node) as well as non-draining nodes (cecal node) were examined for gene expression and local responses to the OVA gene. 14 days after AdOVA administration, spleen cells were also collected for measurement of cytokine production and determination of antigen-specific CTL activities using specific OVA peptide (SIINFEKL) pulsed target cells (EL4 based). Mice deficient in CD8 T cells (CD8 KO) were used to investigate the role of CD8 T cells in the CTL response and protection from tumour challenge.

### **Results:**

1. Staining for  $\beta$ -galactosidase showed that the expression of the transferred gene was widespread across the crypts and villi of the colo-rectal epithelial layer from one to three days post iR administration (Fig 1, 4, 5). The transgene expression was dose dependent (Fig 6).
2. 14 days after AdOVA iR immunization, mice were protected from tumor challenge with OVA-antigen expressing tumour cells (EG7-OVA) either delivered to a systemic site, subcutaneously (Fig 2, 9, 10), or to a local site, intra-mucosally (Fig 11). The protection from tumour challenge after iR immunization was shown to be CD8 dependent through the use of CD8 KO mice where the protection was abolished (Fig 12). Protection was not seen when OVA protein alone was delivered to the rectal tissue (Fig 12).
3. OVA-specific systemic (spleen) and local (Draining lymph nodes) CTL responses were detected 14 days after AdOVA iR immunization (from 20% to 60% in separate experiments vs. ~1% in the control) (Fig 3, 7, 8).
4. The production of INF- $\gamma$ , but not IL-4, was dramatically increased in the culture of spleen cells re-stimulated with OVA protein (1076.7 pg/ml vs. 42.8 pg/ml).

**Conclusions:**

1. Transgenes can be effectively delivered by AdV in the lower GI tract and expressed widespread across the crypts and villi of the colo-rectal mucosal surfaces.
2. Adenovirus-based mucosal intra-rectal (iR) gene delivery induces both strong systemic and local mucosal immune responses, which are antigen specific. This method offers direct advantages as a vaccination route to induce local immune responses within the colo-rectal tissue and within the common mucosal tissue in general. This route of immunization also offers induction of protective CTL activity and long-lasting immune protection from tumour challenge.



Fig 1. LacZ expression visualized in the colo-rectum one day after AdLacZ iR delivery.

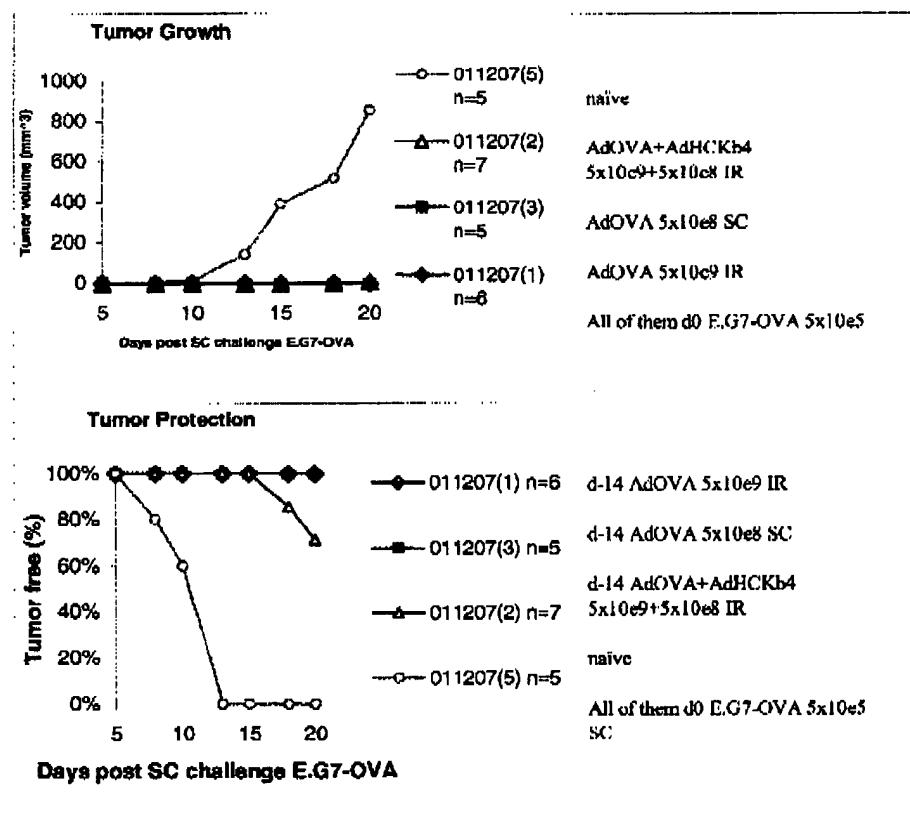


Fig 2. Tumor protection and tumor growth after E.G7-OVA challenge in iR AdOVA-immunized mice.

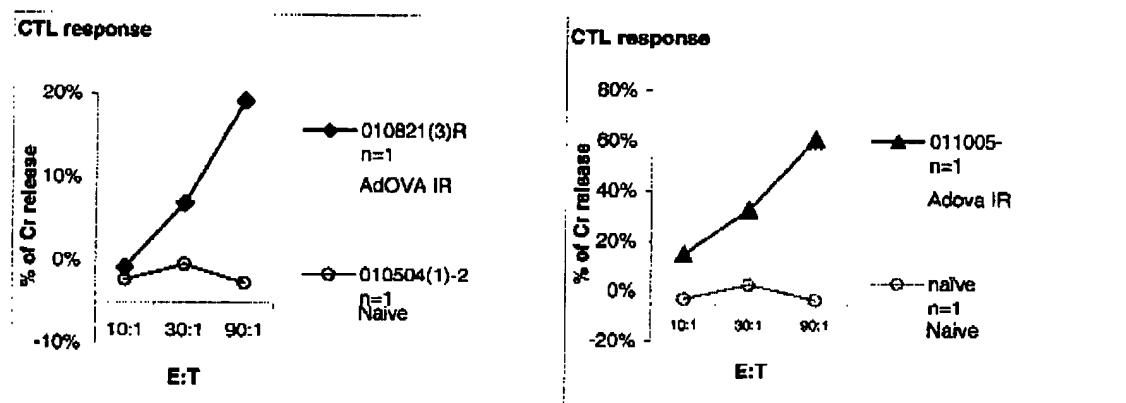
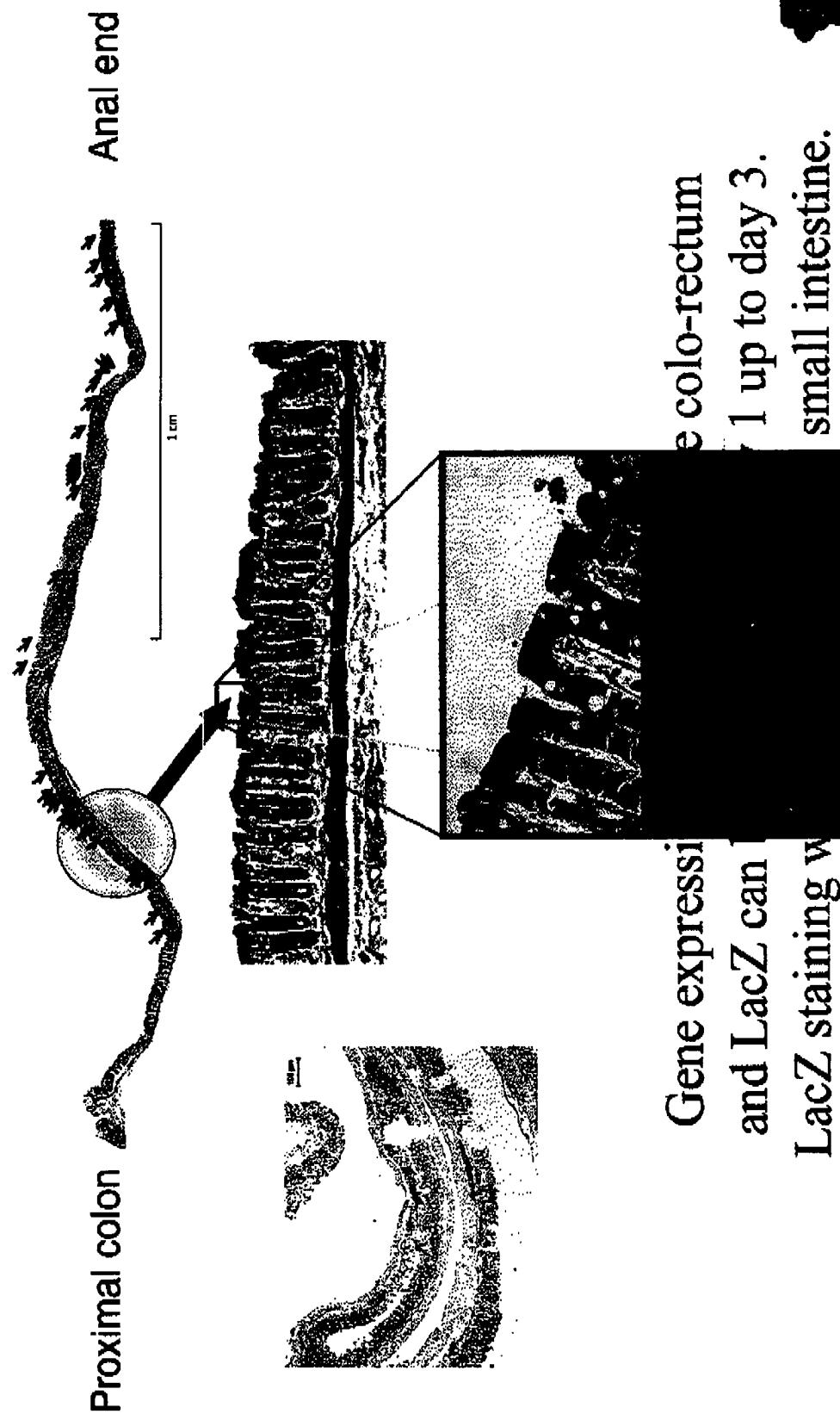


Fig 3. Spleen CTL response against EL-4 pulsed with OVA peptide SIINFEKL. The results represent the CTL response from 2 independent experiments.

# Visualization of gene expression in the colon

Day 2 after AdLacZ  $5 \times 10^9$  pfu IR

Fig 4



Gene expression  
and LacZ can  
be colo-rectum  
1 up to day 3.  
small intestine.

# Visualization of gene expression in the colon

Fig 5

Day 2 after AdLacZ  $5 \times 10^9$  pfu IR



Anti-LacZ Ab  
AdLacZ staining

Gene expression is found across the villi and crypts of the epithelium and “maybe the LP” of the colon, implying efficiency of gene delivery by Adv at high doses.

# Visualization of gene expression in the colon

--- Dose dependent study -----

Fig 6

Day 3 after AdLacZ IR

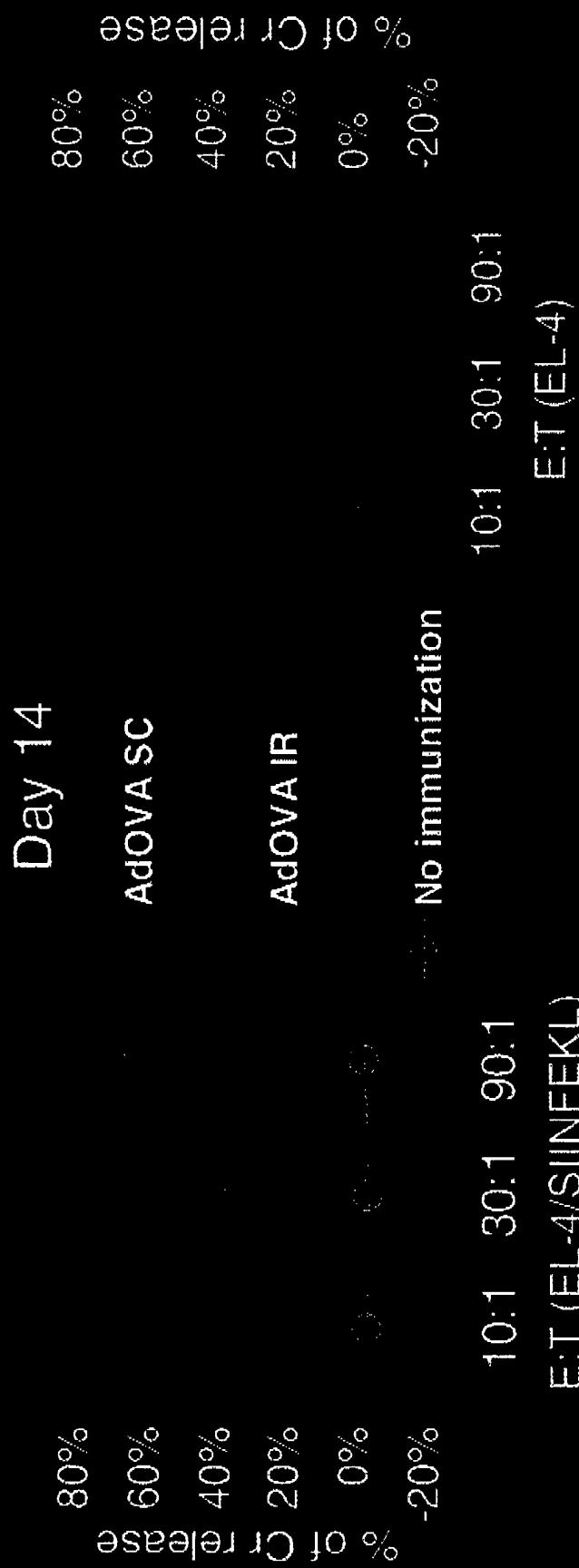


$5 \times 10^8$  pfu       $1 \times 10^9$  pfu       $2 \times 10^9$  pfu

Adv IR-administered gene can be delivered onto the epithelial cells and be expressed.



# Induction of cellular immune responses by AdOVA IR ---- Systemic (spleen)---

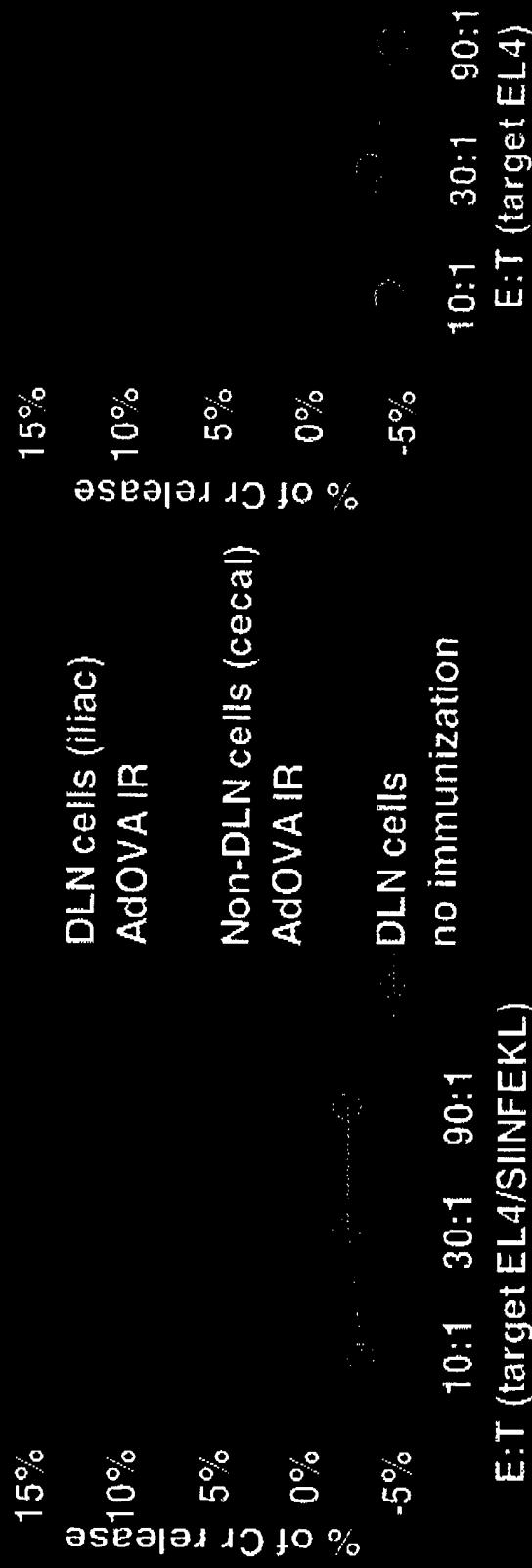


Systemic CTL responses induced by AdOVA IR were antigen-specific.

Fig 7

## Induction of cellular immune responses after AdOVA IR

--- Local (DLNs) ---  
Day 5

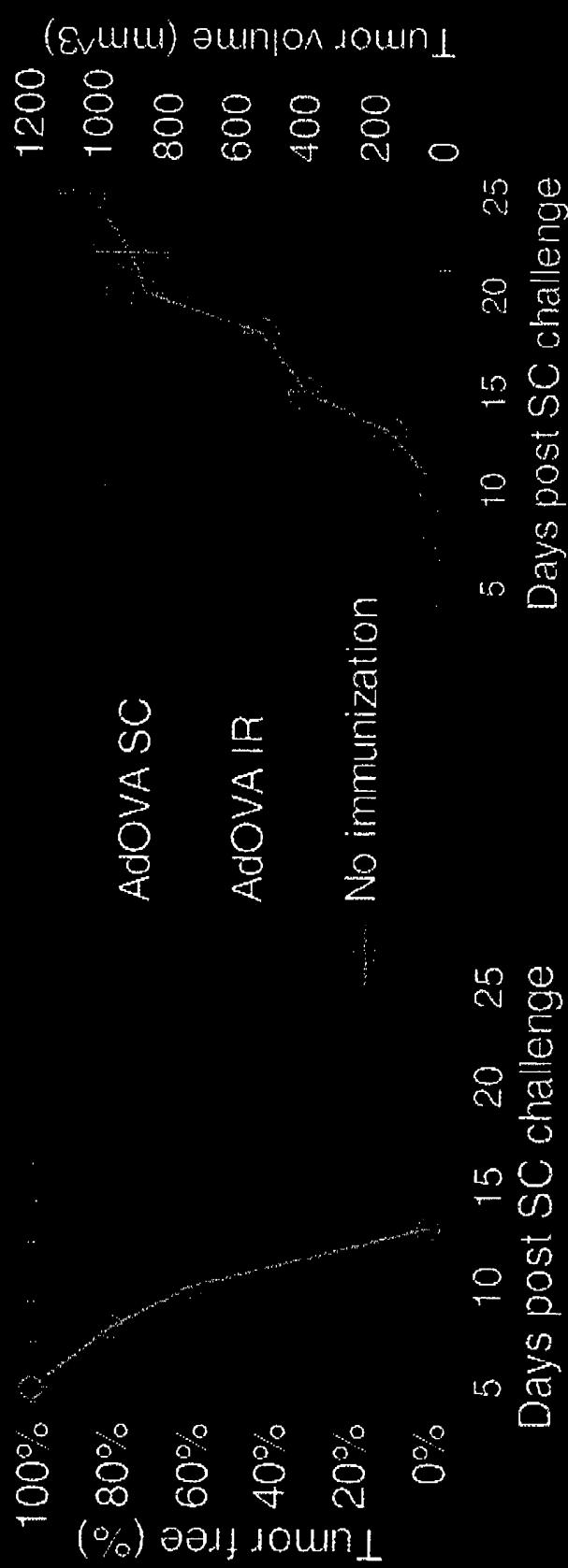


Local DLN primary CTL responses induced by AdOVA IR  
were also antigen-specific.

Fig 8

Induction of protective immunity by AdOVA IR  
--- Systemic immunity against tumor challenge ---

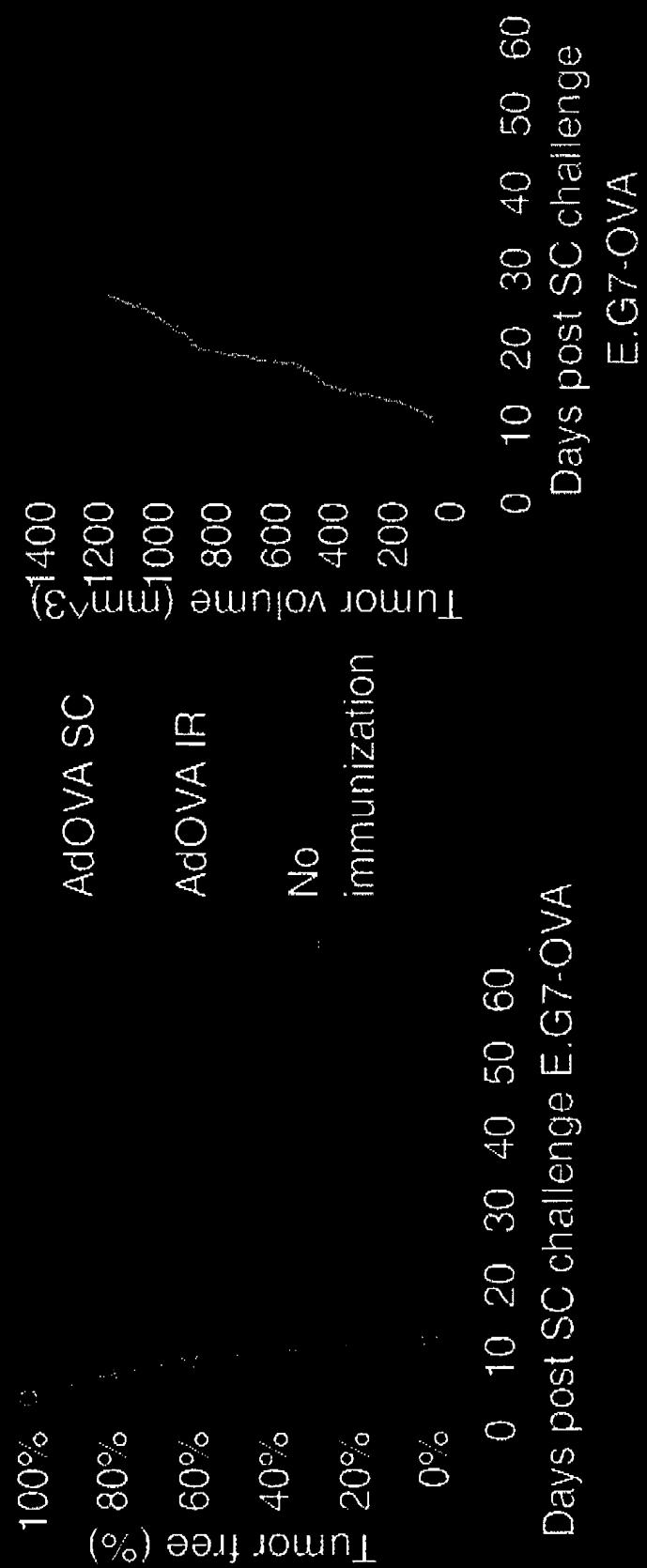
Short term



Systemic immune responses induced by AdOVA were protective

Fig 9

Induction of protective immunity by AdOVA/IR  
--- Systemic immunity against tumor challenge ---  
Long term

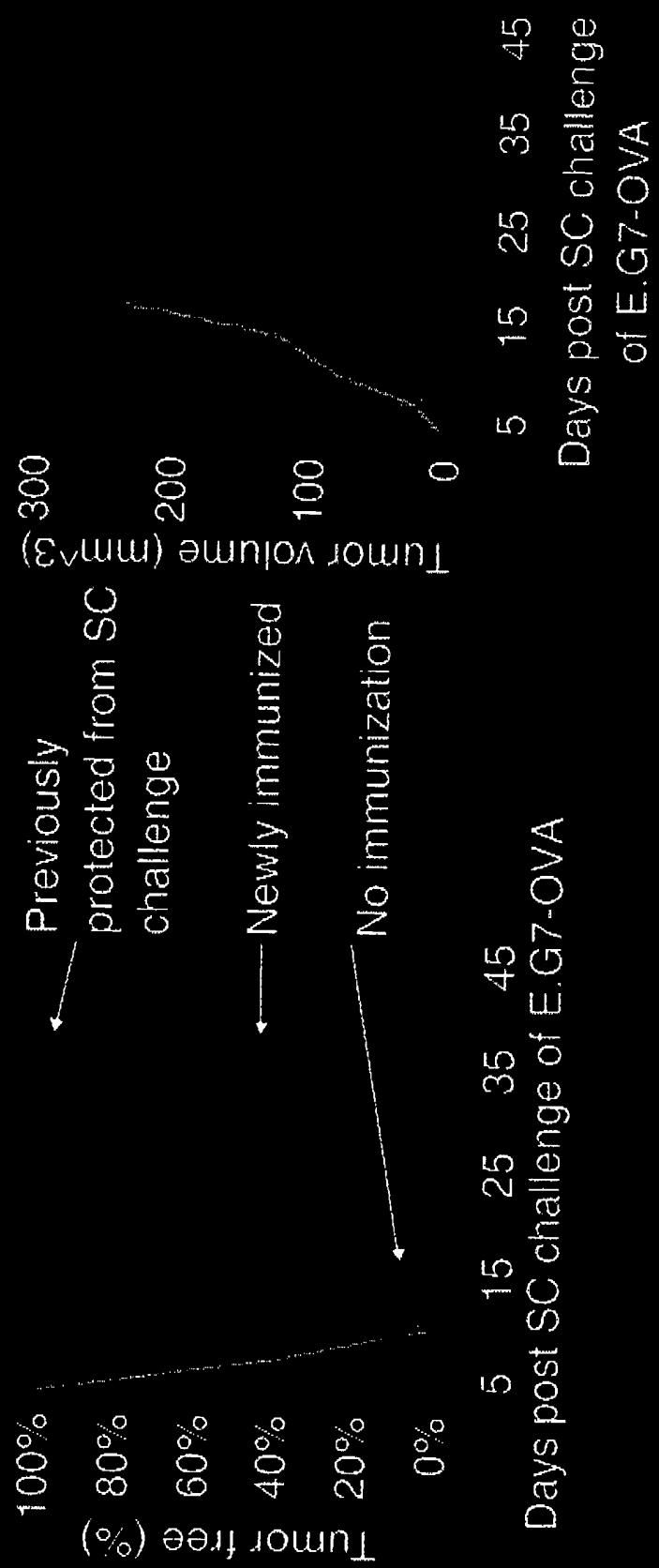


Systemic immune responses induced by AdOVA were protective

Fig 10

# Induction of protective immunity by AdOVA IR

## Local immunity against tumor challenge in the rectal mucosa

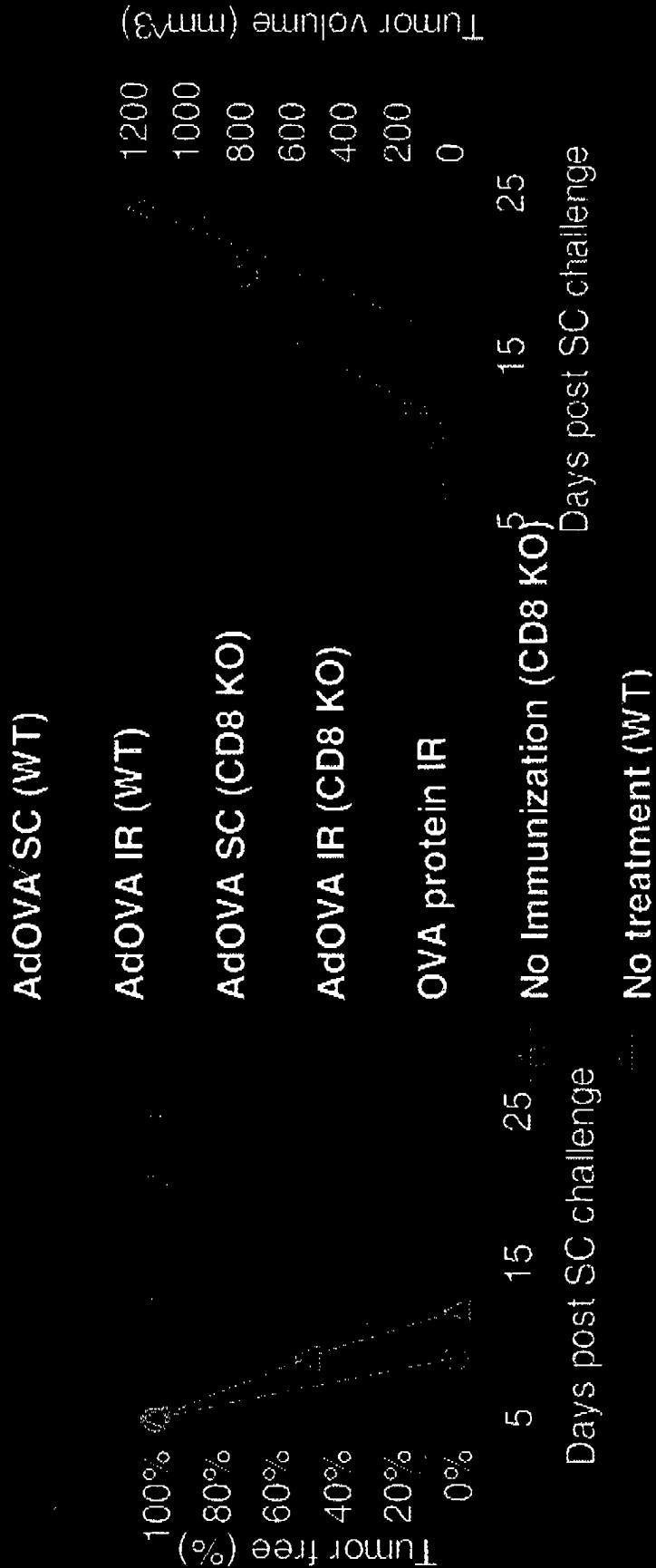


Mucosal immune responses induced by AdOVA were protective

Fig 11

## Induction of protective immunity by AdOVA/IR

-- CD8 dependent --



The CTL responses are CD8 dependent (CD8 KO not protected). Also, OVA protein alone was not able to induce protective immune responses

Fig 12